Osteoarthritis and Cartilage (2001) **9,** 447–453
© 2001 OsteoArthritis Research Society International
doi:10.1053/joca.2000.0411, available online at http://www.idealibrary.com on IDEAL®
1063–4584/01/050447+07 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Effect of load on articular cartilage matrix and the development of guinea-pig osteoarthritis

L. Wei*†, A. Hjerpe‡, B. H. Brismar*† and O. Svensson*†
**Department of Orthopaedic Surgery, †Clinical Research Center, and ‡Department of Pathology, Huddinge University Hospital, Karolinska Institutet, S-141 86 Stockholm, Sweden*

Summary

Objective: To study the biochemical changes in the early development of primary guinea-pig knee osteoarthritis (OA) and its dependence on load.

Methods: Load distribution was modified with below-knee amputation or femur valgus osteotomy in 9-month-old guinea-pigs. Soft tissue sham operated animals served as controls. The composition of uncalcified and calcified articular cartilage at the medial and lateral tibial condyle was studied by analysing small and large proteoglycans (PG) by gel electrophoresis and sulfation pattern with high-performance liquid chromatography. Collagen concentration was also determined.

Results: The articular surfaces with a presumed higher load after surgery had a slight, but consistent, higher water content. Decreased load—on the ipsilateral medial condyle after femur osteotomy, and on the ipsilateral medial and lateral condyles following tibia amputation—was associated with an increased concentration of PGs, while this concentration decreased in condyles with increased load. Collagen concentration followed a similar pattern in the osteotomy group. In the amputated animals collagen concentration went down in all condyles, regardless of change of load. The aggregability and proportion of large and small PGs, the concentration of hyaluronan and the sulfation pattern of chondroitin sulfate was not affected by load. No consistent changes in PG, collagen or HA concentration, HA aggregability or sulfation pattern were seen in the calcified cartilage.

Conclusions: Primary guinea-pig knee OA is a reproducible model similar to human OA. It develops slowly and biochemical changes seem to appear before the morphological lesions become evident. The biochemical events are affected by load redistribution and correlate closely to morphological changes. These changes eventually result in a cartilage devoid in aggrecan, as also has been demonstrated in advanced human OA. All of this makes primary guinea-pig OA a suitable model for studying early OA changes. © 2001 OsteoArthritis Research Society International

Key words: Osteoarthritis, Load, Guinea-pig, Proteoglycans.

Introduction

Invasive studies of the early stages of primary human osteoarthritis (OA) are not feasible for ethical reasons. Therefore, a number of animal models have been designed, most of them involving a graded injury, mimicking clinical situations known to induce secondary OA, e.g. knee joint instability after transection of the anterior cruciate ligament[1–5] or meniscectomy[6–8]. In other models, arthropathy is induced more indirectly by either osteotomies shifting load patterns[9–11], strenuous exercise[12] or repetitive impulse loading[13].

Hartley guinea-pigs develop a spontaneous knee arthropathy similar to primary human OA[14,15]. The lesions progress slowly with age and increasing body weight and their occurrence parallels the presumed distribution of load over the joint—first occurring in the non-meniscus covered part of the medial tibial condyle—in harmony with the notion that mechanical load is a major factor in development of primary OA[13]. Before guinea-pig OA becomes morphologically evident there is an initial reparative or reactive condition, when an increased amount of organic matrix, i.e. PGs and collagen, is laid down. When the cartilage fibrillation becomes morphologically manifest these concentrations decrease[16], as also has been described in advanced human OA[17]. The decreased PG concentration in guinea-pigs is primarily due to decreased synthesis, while, unlike some more rapidly developing secondary OA models[18–20], the degradation is virtually unaffected[21]. An initial increased PG and collagen synthesis has also been reported following transection of the anterior cruciate ligament in dog, referred as a hypertrophic state[18,22–24], while the opposite has been found after strenuous exercise[12,25,26].

Load is an important factor in OA development. By altering load distribution with a unilateral below-knee

Received 5 January 2000; revision requested 22 April 2000; revision received 5 September 2000; accepted 19 December 2000; published online 2 April 2001.

This study was supported by the Swedish Association against Rheumatism, King Gustaf V's 80-year Anniversary Foundation, Loo and Hans Ostermans Foundation for Medical Research, Swedish Medical Research Council (Nos. K 97-17X 12231-01 and K98-17X-12231-02A), Gun and Bertil Stohne's Foundation, Ulla and Gustaf af Ugglas' Foundation, Crafoord's Foundation, Sigurd and Elsa Goljes Memorial Foundation, Swedish Medical Association, Swedish Institute, Ragnhild and Einar Lundströms Memorial Foundation.

Address correspondence to: Anders Hjerpe, M.D., Ph.D., Division of Pathology, F42, Huddinge University Hospital, S-141 86 Stockholm, Sweden. Tel: +46-8-58581017; Fax: +46-8-58581025; E-mail: anders.hjerpe@pathlab.hs.sll.se

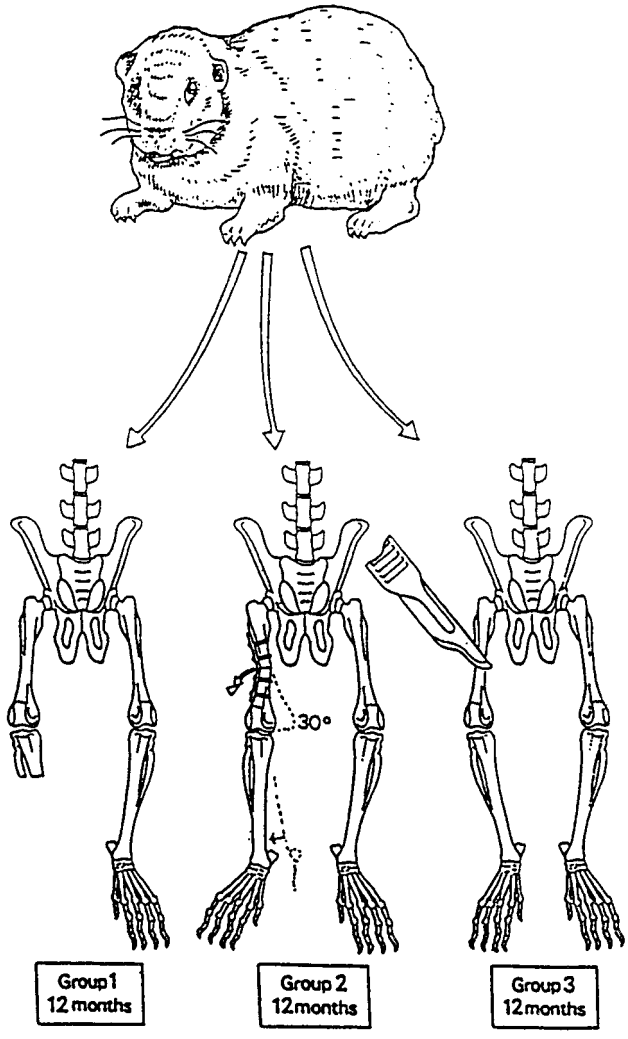

Fig. 1. Experimental design.

amputation, load is transferred from the operated to the contralateral side. A less dramatic change can be induced by valgus femur osteotomy, whereby the distribution of load is shifted from the medial to the lateral condyle. This influences the development of the structural lesions in guinea-pig OA[27] as well as in humans[28,29]. The aim of this study was to see how biochemical changes correlated to lesions of varying severity, testing the hypothesis of graded detoriation of matrix components with advancing lesions and clarifying how early such biochemical changes can be demonstrated during OA development.

Material and methods

Nine-month-old outbred male Hartley guinea-pigs, 1.1±0.08 kg (Møllegård, Copenhagen, Denmark) were randomized into three groups (N=8): group 1 had a unilateral below-knee amputation; group 2 a mid-femoral 30°-valgus osteotomy, internally fixed with a pre-bent stainless steel plate and screws (AO, Davos, Switzerland); group 3, controls, a soft tissue sham operation (Fig. 1). The animals were anesthetized with intramuscular injections of atropine, diazepam and fentanyl-fluanison[16]. Radiographs were taken post-operatively and at 3 months after surgery. After operation, the animals were allowed free mobilization in separate cages. They were killed after 3 months.

All reagents used were of analytical grade. Chondroitin-sulfate (grade II preparation), chondroitinase AC, chondroitin ABC and disaccharide standards were obtained from Sigma (St Louis, MO, U.S.A.). The hyaluronan (HA) standard was from Pharmacia-Upjohn (Healon®, Uppsala, Sweden).

The proximal tibiae were removed and an additional dissection was performed under a dissection microscope. Each condyle was divided into two areas: one central non-meniscus covered and one peripheral meniscus covered. The uncalcified cartilage was separated from the calcified at the tidemark, a well-defined border through the hardness of the underlying mineralized tissue and its opaque appearance in the dissection microscope. Altogether eight fractions from each animal were thus collected (four uncalcified and four calcified) and pooled with the corresponding ones of the other animals in the group to give 15–20 mg of wet tissue in each group. The tissue specimens were dissected, weighted (wet weight) and immediately frozen at −20°C. The tissue was then cut into 20 μm thick slices on a cryostat, lyophilized at −50°C for 24 h, and the dry weight was determined. The PGs were extracted at 4°C for 18 h with 4 M guanidine hydrochloride (GuHCl), containing 0.01 M EDTA and protease inhibitors, using 2×40 μl per mg/dry weight[30]. The calcified cartilage fractions were subsequently extracted with a second GuHCl solution, increasing the EDTA concentration to 0.25 M. The extract obtained from the calcified tissue, using a low concentration of EDTA, was used to estimate the amount of admixture from non-mineralized tissue, assuming similar PG contents as in the overlying non-mineralized tissue, while the extract with a higher concentration of EDTA was considered representative of the mineralized matrix[30]. Residues not extracted were digested with papain and aliquots of these digests were hydrolyzed in 6 M HCl for 18 h and analysed for hydroxyproline contents[31].

Large and small PGs were separated electrophoretically[32]. The aggregability of PG monomers was monitored by incubating aliquots with hyaluronan (HA) before electrophoresis and comparing the mobility with other aliquots, in which the HA binding capacity had been blocked by reduction[32]. The gels were stained with toluidine blue and the distribution was scanned with a Shimadzu Dual-Wavelength Chromato-Scanner, Model CS-930 (Tokyo), the result was evaluated using densitometry.

After incubation of aliquots with chondroitinase AC and ABC sulfation patterns were monitored by high performance liquid chromatography (HPLC) using external standards[33]. Contents of CS and HA was obtained by further digestion with chondroitinase-4 and chondroitinase-6 sulfatases followed by ion suppression HPLC[34].

To monitor procedure reproducibility, the chemical analyses were performed in triplicate.

Results

After osteotomy, the animals moved about unhindered after wound healing. Bone healing was confirmed radiologically (Fig. 2). The amputated animals initially had restricted movement, but they were soon able to walk tripedally. At 12 months of age, all animals appeared to be unimpaired with a normal knee passive range of motion.

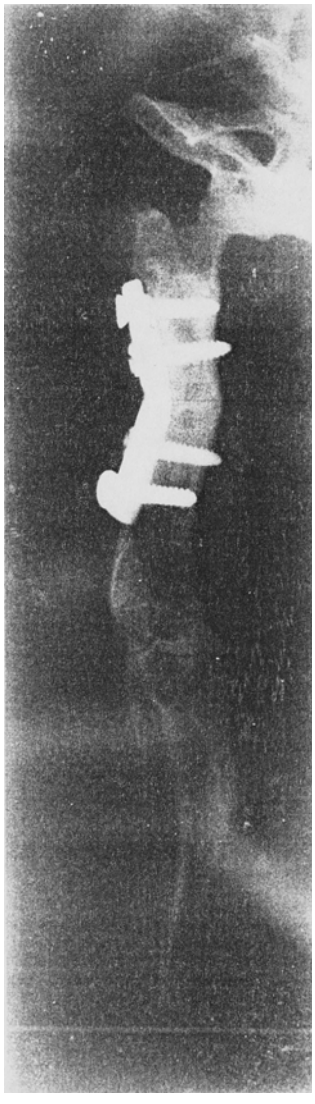

Fig. 2. Radiograph of a guinea-pig hind-limb 12 weeks after 30° mid-femoral valgus osteotomy.

UNCALCIFIED CARTILAGE

Articular surfaces with a presumed higher load after surgery (Table I) had a slight, but consistent higher water content. Decreased load—on the ipsilateral medial condyle after osteotomy, and on the ipsilateral medial and lateral condyles following tibia amputation—was associated with an increase in PG concentration compared with corresponding condyles in sham operated animals (Table II). Condyles with increased load had a decreased PG concentration. Collagen concentration followed a similar pattern in the osteotomy group. In the amputated animals collagen concentration went down regardless change of load (Table III).

The extracted PGs were separated by electrophoresis into one smaller, faster moving band, and two major, closely migrating bands with lower mobility. The faster moving band migrated slightly more slowly than the free CS chains, similarly to decorin and biglycan, while the mobility of the large PG was similar to that of chondrosarcoma aggrecan. The mobility of the PG populations was not changed. The HA concentration did not seem to be correlated to load changes, the concentration was, however, consistently lower in the lateral chondyle cartilage (Table IV). On incubation with HA, about 60% of large PGs aggregated, as seen by the reduced migration at the subsequent electrophoresis. The aggregability was not affected by variations in load (Table V).

MINERALIZED CARTILAGE

The mineralized cartilage contained much lower concentrations of PGs, HA and collagen than its uncalcified counterparts, but there were no consistent load patterns. No correlation to load was seen regarding these parameters (Tables II–IV).

In both mineralized and non-mineralized tissue fractions, the CS of the PGs showed a predominance of 6-sulfated disaccharides, with a considerable proportion of 6-sulfated disaccharides, leaving one to two non-sulfated disaccharides per chain, while there were no oversulfated ones (Table VI). The sulfation pattern was not affected by load change.

Discussion

The effects of experimental load alteration highlight the importance of load as a factor in the development of primary guinea-pig OA. Increased load—at the lateral condyle after osteotomy and at the contralateral side to

Table I
Effects of amputation and osteotomy on hydration (% of wet weight) of the tibial articular cartilage in primary guinea-pig OA

	Condyle	Compartment	Amputation ipsilateral side Low load	Osteotomy medial condyle	Control (sham)	Osteotomy lateral condyle	Amputation contralateral side High load
Uncalcified cartilage	Medial	Central	67 (96)*	63 (90)*	70 (100)*	—	74 (106)*
	Medial	Peripheral	68 (99)	60 (87)	69 (100)	—	76 (111)*
	Lateral	Central	66 (98)	—	68 (100)	69 (102)*	70 (104)*
	Lateral	Peripheral	62 (95)	—	65 (100)	67 (103)	73 (111)
Calcified cartilage	Medial	Central	35 (102)	36 (103)	35 (100)	—	38 (109)
	Medial	Peripheral	33 (105)	32 (102)	31 (100)	—	30 (97)
	Lateral	Central	33 (103)	—	32 (100)	31 (98)	33 (102)
	Lateral	Peripheral	31 (102)	—	31 (100)	29 (95)	30 (98)

*Tissue with morphological OA.
The figures are arranged according to the presumed order of load, least to the left, maximal to the right. Figures within parentheses indicate the value in relation (%) to the corresponding control tissue.

Table II

Effects of amputation and osteotomy on the content of proteoglycans (expressed as μg uronic acid/mg tissue dry weight) in the different fractions and in the groups, cf legend to Table I

	Condyle	Compartment	Amputation ipsilateral side Low load	Osteotomy medial condyle	Control (sham)	Osteotomy lateral condyle	Amputation contralateral side High load
Uncalcified cartilage	Medial	Central	22 (119)*	22 (123)*	18 (100)*		16 (87)*
	Medial	Peripheral	16 (105)	16 (101)	15 (100)		14 (88)*
	Lateral	Central	23 (111)		21 (100)	17 (84)*	20 (91)*
	Lateral	Peripheral	16 (112)		14 (100)	13 (89)	12 (85)
Calcified cartilage	Medial	Central	1.1 (131)	2.9 (346)	0.8 (100)		0.5 (65)
	Medial	Peripheral	0.9 (61)	2.3 (160)	1.4 (100)		2.6 (181)
	Lateral	Central	0.6 (47)		1.2 (100)	1.7 (139)	2.7 (228)
	Lateral	Peripheral	0.5 (45)		1.1 (100)	1.4 (127)	2.4 (218)

*Tissue with morphological OA.

Table III

Effects of amputation and osteotomy on the collagen content of upper tibial articular cartilage (hydroxyproline expressed as μg/mg dry tissue weight)

	Condyle	Compartment	Amputation ipsilateral side Low load	Osteotomy medial condyle	Control (sham)	Osteotomy lateral condyle	Amputation contralateral side High load
Uncalcified cartilage	Medial	Central	68 (95)*	84 (117)*	72 (100)*		55 (77)*
	Medial	Peripheral	56 (68)	80 (97)	83 (100)		79 (96)*
	Lateral	Central	74 (93)		80 (100)	68 (85)*	67 (84)*
	Lateral	Peripheral	60 (86)		70 (100)	62 (90)	61 (88)
Calcified cartilage	Medial	Central	25 (111)	31 (137)	23 (100)		21 (92)
	Medial	Peripheral	23 (106)	25 (117)	22 (100)		21 (97)
	Lateral	Central	20 (92)		22 (100)	20 (92)	22 (97)
	Lateral	Peripheral	17 (94)		19 (100)	19 (103)	20 (106)

*Tissue with morphological OA.
The figures are arranged according to the presumed order of load, cf legend to Table I.

Table IV

The HA concentration (expressed as μg uronic acid/mg tissue dry weight) and ratio of PG/HA (μg uronic acid/μg uronic acid)

	Condyle	Compartment	Amputation ipsilateral side		Osteotomy medial condyle		Control (sham)		Osteotomy lateral condyle		Amputation contralateral side	
			HA	PG/HA	HA	PG/HA	HA	PG/HA	HA	PG/HA	HA	PG/HA
			Low load								High load	
Uncalcified cartilage	Medial	Central	0.64	34	0.60	35	0.62	30			0.57	28
	Medial	Peripheral	0.55	30	0.51	31	0.53	30			0.43	32
	Lateral	Central	0.46	51			0.49	43	0.44	40	0.47	41
	Lateral	Peripheral	0.45	36			0.43	34	0.39	33	0.41	30
Calcified cartilage	Medial	Central	0.02	55	0.05	58	0.02	39			0.04	14
	Medial	Peripheral	0.02	44	0.04	58	0.03	48			0.05	52
	Lateral	Central	0.01	56			0.02	51	0.03	58	0.05	51
	Lateral	Peripheral	0.01	50			0.02	49	0.03	47	0.04	60

The figures are arranged according to the presumed order of load, cf legend to Table I.

amputation—speeded up the process[27]. In the present study the reduced PG concentration correlated closely to the stereologically estimated degree of fibrillation (Fig. 3). Increased load—at the lateral condyle after osteotomy and at the contralateral side to amputation—resulted in a decreased PG and collagen concentration, probably due to decreased synthesis activity as seen at 12 months on the medial tibial condyle in non-operated animals[21]. Decreased load—on the medial condyle after osteotomy—seemed to alter this process leading to an increased PG and collagen concentration and a reduced fibrillation index (Fig. 3). Bendele and Hulman[35] showed in a diet experiment that the severity of guinea-pig OA is related to body mass. Also in other model systems, load reduction retards and diminishes cartilage destruction[10,28,29]. Below-knee amputation slowed down the progression of guinea-pig OA as well, but did not completely stop the development of OA[27]. Perhaps irreversible lesions have developed already

Table V
The large and small proteoglycans in uncalcified cartilage (expressed as μg uronic acid/mg tissue dry weight) and aggregability of proteoglycan monomers with hyaluronic acid

Condyle	Compartment	Amputation ipsilateral side			Osteotomy medial condyle			Control (sham)			Osteotomy lateral condyle			Amputation contralateral side		
		LPG	SPG	Agg %	LPG	SPG	Agg %	LPG	SPG	Agg %	LPG	SPG	Agg %	LPG	SPG	Agg %
		Low load												High load		
Medial	Central	20	1.7	55	20	1.8	62	17	1.5	62				15	1.3	58
Medial	Peripheral	15	1.3	54	15	1.3	62	14	1.3	62				13	1.1	57
Lateral	Central	21	1.9	56				19	1.7	64	16	1.4	61	18	1.5	51
Lateral	Peripheral	15	1.3	55				13	1.2	65	12	1.0	60	11	1.0	58

LPG, large proteoglycans; SPG, small proteoglycans; Agg, aggregability.
The figures are arranged according to the presumed order of load, cf legend to Table I.

Table VI
The sulfation pattern of chondroitin sulfate (ratios of 0S:6S:4S)

	Condyle	Compartment	Amputation ipsilateral side 0S:6S:4S Low load	Osteotomy medial condyle 0S:6S:4S	Control (sham) 0S:6S:4S	Osteotomy lateral condyle 0S:6S:4S	Amputation contralateral side 0S:6S:4S High load
Uncalcified cartilage	Medial	Central	0.3:2.3:1	0.2:2.1:1	0.2:2.0:1		0.2:1.8:1
	Medial	Peripheral	0.2:1.8:1	0.2:2.1:1	0.2:1.8:1		0.2:1.8:1
	Lateral	Central	0.2:1.9:1		0.2:2.0:1	0.2:1.9:1	0.2:1.9:1
	Lateral	Peripheral	0.3:1.7:1		0.2:1.6:1	0.2:1.6:1	0.2:1.8:1
Calcified cartilage	Medial	Central	0.6:1.5:1	0.4:1.1:1	0.3:1.0:1		0.5:1.0:1
	Medial	Peripheral	0.7:1.4:1	0.2:1.0:1	0.2:1.0:1		0.2:1.0:1
	Lateral	Central	0.5:1.3:1		0.2:1.1:1	0.1:1.0:1	0.2:1.0:1
	Lateral	Peripheral	0.6:1.2:1		0.2:1.1:1	0.2:0.9:1	0.2:1.0:1

The figures are arranged according to the presumed order of load, cf legend to Table I.

at 9 months? Interestingly, the development of fibrillation in the amputated limbs was seen in parallel with reduced bone volume. Subchondral bone thickening may not be a pre-requisite for the development of primary OA as previously has been suggested[13].

Both condyles on the amputated limb showed decreased collagen concentrations, differing from the pattern seen on the medial condyle following osteotomy. Load redistribution in the unloaded knee is, though, rather extreme and the biochemical mechanisms may not be comparable to those on the opposite leg or following osteotomy.

The PG changes in the present study are in harmony with those on human OA, where cartilage samples show a progressive loss of glycosaminoglycans in cartilage with increasing histological OA grade[17,36]. The parallel increased hydration[36] is probably due to a failure of the damaged collagen network to oppose the swelling pressure of the PGs[37,38]. Findings of an increased collagenase activity in guinea-pig, human and dog OA cartilages[39,40,41] support this notion.

OA is most likely not a single entity but rather a common end-stage of several different causes. Hence, several animal models have been developed with different metabolic and morphologic characteristics. Most of them involve a changed loading pattern. The hypertrophic remodeling described after anterior cruciate ligament (ACL) transection in dog result in an increased PG and collagen synthesis[23,24], probably representing a reparative or compensating state, resembling findings in pre-arthritic guinea-pig cartilage. In the ACL model, full thickness lesions do not appear until almost 4 years after surgery[4], compared with the more rapidly developing destruction in rabbits following destabilization[1,5]. A drawback of these invasive models, e.g. arthrotomy and partial meniscectomy, is that the surgical procedure may induce unspecific effects. Non-invasive OA models, e.g. osteotomy[9–11] or strenuous exercise[12,25,26] develop slowly and do not seem to progress to full-depth lesions. The relevance of the reduced PG and collagen concentrations found after strenuous exercise is therefore unclear. Repetitive impulse loading, on the other hand, induce more severe cartilage changes, but the histological lesions seem to develop differently to those in guinea-pig OA, the first changes occurring in the mid and deep zones rather than in superficial ones[13].

In the natural history of guinea-pig OA, the concentrations of collagens and PGs in articular cartilage gradually increase between 6 and 9 months[21], i.e. before OA lesions can be detected in the microscope. This higher fixed charge density increases the tissue swelling pressure and may be a response to the increased load. When OA becomes overt, the affected portions of the tissue show drastically reduced concentrations of both PGs and collagen, while the tissue hydration increases in the unloaded tissue[16]. A similar change in matrix composition was also present after osteotomy in the lateral condyle, and in the tibia contralateral to the amputation. The close correlation between structural changes and matrix composition suggests that the development of OA is a lengthy process, in which biochemical changes occur early. The findings accord with the hypothesis that an increased demand to

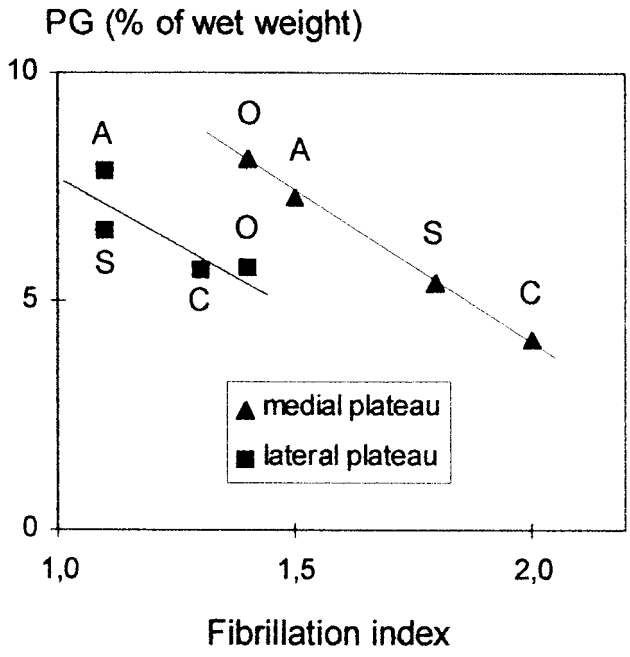

Fig. 3. The concentration of PGs in non-mineralized articular cartilage from the central portions of the tibial condyles correlates with the extent of OA-associated changes estimated with the fibrillation index—i.e., the ratio between the actual surface and its smooth contour. A=amputated side, C=side contralateral to amputation, O=osteotomy and S=sham operation.

carry load induces a compensatory mechanism in the articular cartilage, which increases the PG concentration and thereby its density of fixed charges and its swelling pressure. With a sustained excessive load, the rate of PG synthesis becomes insufficient to maintain a high enough tissue swelling pressure of the matrix[21]. Simultaneously, the concentration of collagen also decreases[16] and the tissue will therefore lose its tensile strength.

The change in concentrations of PGs and collagen occurs early in the sequence of events leading to OA. At 9 months, however, it seems as if the process has become irreversible although there were no substantial morphological signs of OA and the contents of PGs and collagen still were high[16]. It therefore seems that the biochemical changes associated with the development of OA may be preceded by other, as yet unknown, events, perhaps on the cellular level.

References

1. Hulth A, Lindberg L, Telhag H. Experimental osteoarthritis in rabbits. Preliminary report. Acta Orthop Scand 1970;41:522–30.
2. Pond MJ, Nuki G. Experimentally-induced osteoarthritis in the dog. Ann Rheum Dis 1973;32:387–8.
3. Vignon E, Bejui J, Mathieu P, Hartmann JD, Ville G, Evreux JC, *et al*. Histological cartilage changes in a rabbit model of osteoarthritis. J Rheumatol 1987; 14:104–6.
4. Brandt KD, Myers SL, Burr D, Albrecht M. Osteoarthritis changes in canine articular cartilage, subchondral bone and synovium fifty-four months after transection of the anterior cruciate ligament. Arthritis Rheum 1991;34:1560–70.
5. Yoshioka M, Coutts RD, Amiel D, Hacker SA. Characterization of a model of osteoarthritis in the rabbit knee. Osteoarthritis Cart 1996;4:87–98.
6. Moskowitz RW, Howell DS, Goldberg VM, Muniz O, Pita JC. Cartilage proteoglycan alterations in an experimentally induced model of rabbit osteoarthritis. Arthritis Rheum 1979;22:155–63.
7. Colombo C, Butler M, O'Byrne E, Hickman L, Swartzendruber D, Selwyn M, *et al*. A new model of osteoarthritis in rabbits. I. Development of knee joint pathology following lateral meniscectomy and section of the fibular collateral and sesamoid ligaments. Arthritis Rheum 1983;26:875–86.
8. Little C, Smith S, Ghosh P, Bellenger C. Histomorphological and immunohistochemical evaluation of joint changes in a model of osteoarthritis induced by lateral meniscectomy in sheep. J Rheumatol 1997;24:2199–209.
9. Johnson RG, Poole AR. Degenerative changes in dog articular cartilage induced by a unilateral tibial valgus osteotomy. Exp Pathol 1988;33:145–64.
10. Wu DD, Burr DB, Boyd RD, Radin EL. Bone and cartilage changes following experimental varus and valgus angulation. J Orthop Res 1990;8:572–85.
11. Panula HE, Helminen HJ, Kiviranta I. Slowly progressive osteoarthritis after tibial valgus osteotomy in young beagle dogs. Clin Orthop 1997;(343): 192–202.
12. Kiviranta I, Tammi M, Jurvelin J, Arokoski J, Saamanen AM, Helminen HJ. Articular cartilage thickness and glycosaminoglycan distribution in the canine knee joint after strenuous running exercise. Clin Orthop 1992;(283):302–8.
13. Radin EL, Martin RB, Burr DB, Caterson B, Boyd RD, Goodwin C. Effects of mechanical loading on the tissues of the rabbit knee. J Orthop Res 1984;2: 221–34.
14. Bendele AM, Hulman JF. Spontaneous cartilage degeneration in guinea-pigs. Arthritis Rheum 1988; 31:561–5.
15. De Bri E, Reinholt FP, Svensson O. Primary osteoarthrosis in guinea-pigs: a stereological study. J Orthop Res 1995;13:769–76.
16. Wei L, Svensson O, Hjerpe A. Correlation of morphological and biochemical changes in the natural history of the spontaneous osteoarthrosis in guinea-pigs. Arthritis Rheum 1997;40:2075–83.
17. Thompson RC Jr, Oegema TR Jr. Metabolic activity of articular cartilage in osteoarthritis. An in vitro study. J Bone Joint Surg (Am) 1979;61:407–16.
18. Carney SL, Billingham ME, Muir H, Sandy JD. Demonstration of increased proteoglycan turnover in cartilage explants from dogs with experimental osteoarthritis. J Orthop Res 1984;2:201–6.
19. Ratcliffe A, Billingham ME, Saed-Nejad F, Muir H, Hardingham TE. Increased release of matrix components from articular cartilage in experimental canine osteoarthritis. J Orthop Res 1992;10:350–8,.
20. Axelsson S, Bjornsson S, Holmlund A, Hjerpe A. Metabolic turnover of sulfated glycosaminoglycans and proteoglycans in rabbit temporomandibular joint cartilages with experimentally induced osteoarthrosis. Acta Odont Scand 1994;52:65–71.
21. Wei L, Svensson O, Hjerpe A. Proteoglycan turnover during development of spontaneous osteoarthrosis in guinea-pigs. Osteoarthritis Cart 1998;6:410–16.

22. Adams ME, Brandt KD. Hypertrophic repair of canine articular cartilage in osteoarthritis after anterior cruciate ligament transection. J Rheumatol 1991;18: 428–35.
23. Venn G, Billingham ME, Hardingham TE. Increased proteoglycan synthesis in cartilage in experimental canine osteoarthritis does not reflect a permanent change in chondrocyte phenotype. Arthritis Rheum 1995;38:525–32.
24. Eyre DR, McDevitt CA, Billingham ME, Muir H. Biosynthesis of collagen and other matrix proteins by articular cartilage in experimental osteoarthrosis. Biochem J 1980;188:823–37.
25. Saamamen AM, Kiviranta I, Jurvelin J, Helminen HJ, Tammi M. Proteoglycan and collagen alterations in canine knee articular cartilage following 20 km daily running exercise for 15 weeks. Connect Tissue Res 1994;30:191–201.
26. Arokoski J, Kiviranta I, Jurvelin J, Tammi M, Helminen HJ. Long-distance running causes site-dependent decrease of cartilage glycosaminoglycan content in the knee joints of beagle dogs. Arthritis Rheum 1993;36:1451–9.
27. Wei L, de Bri E, Lundberg A, Svensson O. Mechanical load and primary guinea-pig osteoarthrosis. Acta Orthop Scand 1998;69:351–7.
28. Bergenudd H, Johnell O, Redlund-Johnell I, Lohmander LS. The articular cartilage after osteotomy for medial gonarthrosis. Biopsies after 2 years in 19 cases. Acta Orthop Scand 1992;63: 413–16.
29. Odenbring S, Egund N, Lindstrand A, Lohmander LS, Willén H. Cartilage regeneration after proximal tibial osteotomy for medial gonarthrosis. Clin Orthop 1992;277:210–16.
30. Franzén A, Heinegård D. Characterization of proteoglycans from the calcified matrix of bovine bone. Biochem J 1984;224:59–66.
31. Stegeman H, Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967;18:267–73.
32. Björnsson S. Size-dependent separation of proteoglycans by agarose gel electrophoresis. Anal Biochem 1993;210:292–8.
33. Hjerpe A, Antonopoulos CA, Engfeldt B. Determination of sulfated disaccharides from chondroitin sulfates by high-performance liquid chromatography. J Chromatogr 1979;171:339–44.
34. Hjerpe A, Antonopoulos CA, Engfeldt B. The determination of hyaluronic acid using high-performance liquid chromatography of chondroitinase digests. J Chromatogr 1982;245:365–68.
35. Bendele AM, Hulman JF. Effects of body weight restriction on the development and progression of spontaneous osteoarthritis in guinea-pigs. Arthritis Rheum 1991;34:1180–4.
36. Venn M, Maroudas A. Chemical composition and swelling of normal and osteoarthrotic femoral head cartilage. I. Chemical composition. Ann Rheum Dis 1977;36:121–9.
37. Maroudas A, Venn M. Chemical composition and swelling of normal and osteoarthrotic femoral head cartilage. II. Swelling Ann Rheum Dis 1977;36: 399–406.
38. Basser PJ, Schneiderman R, Bank RA, Wachtel E, Maroudas A. Mechanical properties of the collagen network in human articular cartilage as measured by osmotic stress technique. Arch Biochem Biophys 1998;351:207–19.
39. Huebner JL, Otterness IG, Freund EM, Caterson B, Kraus VB. Collagen 1 and collagen 3 expression in a guinea-pig model of osteoarthritis. Arthritis Rheum 1998;41:877–90.
40. Pelletier JP, Martel-Pelletier J, Altman RD, Ghandur-Mnaymneh L, Howell DS, Woessner JF Jr. Collagenolytic activity and collagen matrix breakdown of the articular cartilage in the Pond-Nuki dog model of osteoarthritis. Arthritis Rheum 1983;26:866–74.
41. Pelletier JP, Martel-Pelletier J, Howell DS, Ghandur-Mnaymneh L, Enis JE, Woessner JF Jr. Collagenase and collagenolytic activity in human osteoarthritic cartilage. Arthritis Rheum 1983;26:63–8.